

Review of “Comprehensive analysis of molecular phylogeographic structure among the meadow jumping mice (*Zapus hudsonius*) reveals evolutionary distinct subspecies” by King et al. 2006.

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In this report, the authors investigate the taxonomic relationships among five previously defined subspecies of *Zapus hudsonius* using genetic data. The relationship among these subspecies has recently been called into question by Ramey et al. (2005) who recommended synonymizing three subspecies (*Z. h. campestris*, *Z. h. intermedius*, and *Z. h. preblei*) based on genetic and morphological data. The objective of this study was to provide further genetic information regarding the taxonomic status of these subspecies with a special focus on the relationship among *Z. h. campestris*, *Z. h. intermedius*, and *Z. h. preblei*. This study does not address morphology and thus, I will not address it in this review. In this study, fresh tissue samples were obtained from several sampling locales within each of the five subspecies. King et al. (2006) sequenced two regions of the mitochondria (control region and cytochrome b) and gathered data from 21 nuclear microsatellites. They found that there were no shared mitochondrial DNA haplotypes and significant nuclear genetic structuring among subspecies suggesting that the taxonomic delineations should not be changed, as proposed by Ramey et al. (2005). The authors discuss the differences between their study and the study by Ramey et al. (2005) and provide reasons why they believe their work to be better.

There are several significant differences between the two studies. The study by King et al. (2006) uses more samples (320 samples of *Z. hudsonius* vs. 198 used by Ramey et al. 2005), much more sequence data from two mtDNA regions (total of 1380 base pairs vs. 346 base pairs of only control region mtDNA used by Ramey et al. 2005), and many more microsatellite loci (21 vs. 5 used by Ramey et al. 2005). The two studies use some different methods to analyze their data although some are similar. Perhaps the biggest difference is in sampling design and choice of sampling material. Ramey et al. (2005) collected a small number of samples from many locations throughout the range of each subspecies, while King et al. (2006) collected a large number of samples from a fewer number of sample sites within the range of each subspecies. A sampling design combining both of these strategies would be optimal (i.e. heavily sample sites at more locations that are more representative of the range of each subspecies). The danger in collecting too few samples per site is that when using an analysis based on frequency differences, it is unlikely that the true frequencies of haplotypes or alleles are accurately estimated using this sampling regime. The danger in intensively sampling too few areas is that while you may be accurately estimating haplotype and allele frequencies at certain locations, they may be different at the edges of the range, particularly in species whose movements are limited. King et al. (2006), however, did sample *Z. h. preblei* and *Z. h. campestris* thoroughly, so it is likely that at least within those two subspecies that the allele and haplotype frequencies are relatively well represented. Ramey et al. (2005) extracted DNA from museum skins and some liver tissue (it is not clear how many were skins and how many were liver) while King et al. (2006) used fresh tissue (blood and ear

punches) for their analyses. Given the difficulties associated with DNA extracted from museum specimens and the fact that some samples were dropped from the study by Ramey et al. (2005) because they were likely “misidentified”, it would be nice to see a subset of the DNA from museum specimens genotyped at more microsatellite loci and sequenced using both mitochondrial regions to verify the previous results.

1) Please analyze the techniques used in the population and phylogenetic evaluation of *Z. h. preblei* and other taxa. Were the appropriate methodologies and markers used?

King et al. (2006) sequenced two regions of the mitochondria (control region and cytochrome b) providing 1380 base pairs of sequence data and used 21 nuclear microsatellites. They used appropriate markers and collected an impressive amount of information from the samples collected in their study. Their population and phylogenetic analyses were valid and quite thorough.

2) Based on the data presented in the report, do you support the authors’ conclusions about the taxonomic validity of *Z. h. preblei* and neighboring subspecies?

The data provided in this report are notable in that they show very strong and significant differences among all 5 subspecies. These data suggest a high level of genetic differentiation among subspecies and no real evidence of gene flow among them. This result, however, may be influenced by the sampling design of the study. Because King et al. (2006) sampled a smaller number of sites than Ramey et al. (2005) they may be better representing what the core of each subspecies looks like and not representing as well what may be going on in areas where the subspecies ranges are adjacent. Additionally, because the samples gathered by King et al. (2006) were obtained from mice trapped after emergence from hibernation there may be an increased probability that the sampled individuals were more closely related, which would result in even more distinctness among sampling sites. I feel, however, that King et al. (2006) did do a thorough job of sampling within the *Z. h. preblei* subspecies and sampled reasonably well within *Z. h. campestris*, which were of primary interest to the study. It is likely that *Z. hudsonius* in general do not move very far and that as a result there may be a lot of genetic structure within a subspecies purely due to the species’ biology. This is likely why King et al. (2006) are picking up such strong differences even within a subspecies (in *Z. h. preblei* and in *Z. h. intermedius*).

Avice and Ball (1990) defined a subspecies as “groups of actually or potentially interbreeding populations phylogenetically distinguishable from, but reproductively compatible with, other such groups. Importantly, the evidence for phylogenetic distinction must normally come from the concordant distribution of multiple, independent genetically based traits.” King et al. (2006) found gene flow among subspecies to be negligible at multiple independent loci, and while that may be a bit overemphasized because of their sampling design (i.e. there may be a small amount of haplotype sharing at

the edge of the subspecies range that was not picked up by King et al. (2006)) I would still concur that these are likely good subspecies.

3) Based on the data presented in the report, do you support the authors' conclusions that *Z. h. prebleii* is comprised of at least two distinct population segments worthy of individual management consideration?

The data provided in this report do show two distinct populations of *Z. h. prebleii* that do not share any haplotypes. Further, their STRUCTURE analysis shows that the nuclear data are consistent with the mitochondrial data suggesting that the differences between the two *Z. h. prebleii* populations are not merely a function of sex-biased dispersal. I do feel that one needs to consider the biology of the species and realize that because their dispersal capability is limited, these differences may be inherent to the species although they may also be exacerbated by human encroachment. Having said that, the data by King et al. (2006) do show that there are no shared haplotypes between the north and southern populations of *Z. h. prebleii* suggesting that gene flow is minimal and that it has been for some time.

4) Are there possible alternative interpretations of the data that could be drawn from the genetics data? How likely are these possibilities?

It is possible that *Z. hudsonius* in general exhibit very low levels of gene flow throughout the range and that if one sampled extensively within one subspecies that one might find many distinct "populations" that do not share haplotypes and that are significantly different from one another and that these differences grow stronger the further apart the sampling locales (i.e. isolation by distance). I think that this scenario may be partly true for the species, however, King et al. (2006) did find shared haplotypes within each of the putative subspecies (some sampling sites of which were very geographically distant) and the results from the STRUCTURE analysis really suggest otherwise.

5) What additional analysis, if any, is needed to verify the study's assertions and why?

King et al. (2006) did an excellent job getting the most information out of the samples collected. Additional analyses of these samples would not, in my opinion, change the outcome. The inferences from this study would be stronger if samples were collected from additional sites making the sampling scheme comprehensive and exhaustive in the range of each subspecies.

6) The conclusions of Ramey et al. (2005) and King et al. (2006) would appear to conflict. What are the most likely explanations for this conflict? Has this new information changed your conclusions regarding the synonymization of *Z. h. preblei* and neighboring subspecies?

I have highlighted several differences between the two studies above. The most striking issue about the comparison between these two studies, in my opinion, is that they are so different. King et al. (2006) found no shared haplotypes and very strong differentiation among subspecies, while Ramey et al. (2005) did find shared haplotypes among most of the putative subspecies and lower levels of differentiation. While this could be due to differences in sampling design, I am a little skeptical because of King et al.'s (2006) thorough sampling of *Z. h. preblei* and decent coverage of *Z. h. campestris*. A fundamental aspect of the paper by Ramey et al. (2005) is the fact that haplotypes are shared among the different subspecies. This is particularly the case when comparing *Z. h. campestris* and *Z. h. preblei*. Ramey et al. (2005) found that all four of the haplotypes present in *Z. h. preblei* were also found in five *Z. h. campestris* samples from Custer, SD and two *Z. h. campestris* individuals from Carter, MT. King et al. (2006) also found four haplotypes in *Z. h. preblei* yet although they sampled extensively in Custer, SD, they never found any haplotypes that matched those four haplotypes characteristic of *Z. h. preblei*. In my previous reviews of the two reports by Ramey et al. (which ultimately lead to the publication by Ramey et al. 2005) I discussed the fact that those seven *Z. h. campestris* samples that shared haplotypes with *Z. h. preblei* could perhaps have been mislabeled or misidentified. In their paper, Ramey et al. (2005) dropped certain other samples out of the study based on their genetic haplotypes assuming that they had been misidentified so it is plausible that some kind of error may have occurred, particularly if those samples were from museum skins. It would be extremely informative to have the seven samples reanalyzed using the techniques of King et al. (2006) (e.g. additional sequence data and additional microsatellite loci). Given the discrepancies between the two studies, I would not be supportive of changing the taxonomic delineations without strong evidence suggesting that the strong differentiation reported by King et al. (2006) was merely an isolation by distance effect resulting from limited dispersal capability of *Z. hudsonius*. More intensive sampling across the range of each subspecies (essentially combining both sampling strategies) would provide a better picture of differentiation within and among subspecies. Further, because there is some question about whether there are shared haplotypes among subspecies, validation of key museum specimens is essential.

Literature Cited

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology* 7:45-67.